a. Determination of resting left ventricular EF (%).

NAME alone (60  $\pm$  9 and 55  $\pm$  8%, respectively) and hearts perfused with L-NIL alone (57  $\pm$  9 and 67  $\pm$  4%, respectively).

[000112] Unlike initial pretreatment with rFGF-2, addition of the growth factor to the coronary perfusate after the onset of ischemia, immediately before reperfusion, did not improve LV function 20 min after reperfusion (LVP  $60 \pm 4\%$ ,  $dP/dt_{max}$   $62 \pm 4\%$ , and  $dP/dt_{min}$   $58 \pm 4\%$ , all P = NS vs. control). As in the case of acute ischemic changes, pretreatment with either L-NAME or L-NIL led to a complete inhibition of rFGF-2 effects (Figs.  $\underline{2}$  and  $\underline{3}$ ).

[000113] Isolated Heart Preparation Hearts were excised from adult C57/BL6 mice of either sex that had been anesthetized and heparinized (500 U/100 g body wt). The aorta was slipped over a 20-gauge blunt-tipped stainless steel needle through which oxygenated (95%  $O_2$ -5%  $CO_2$ ) Krebs-Henseleit (KH) buffer (in mM: 118.0 NaCl, 4.7 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.5 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, 23.0 NaHCO<sub>3</sub>, 10.0 dextrose, and 0.3 EDTA, pH 7.4) was pumped at a rate of ~3 ml/min. An intraventricular balloon catheter system specially designed for the mouse heart was passed through the mitral annulus into the left ventricle, and the distal end of the balloon catheter was connected to a Statham P23b (Gould, Cleveland, OH) transducer to record intraventricular pressure. Left ventricular (LV) pressure recordings were analyzed with regard to LV developed pressure (LVP), LV end-diastolic pressure, peak rate of pressure development (dP/d $t_{max}$ ), time to 90% pressure decline, and peak rate of pressure decline (dP/d $t_{min}$ ).

[000114] Ischemia and reperfusion. The hearts were subjected to no-flow ischemia for 15 min. The organ bath was evacuated of its oxygenated solution and refilled with nitrogen-saturated perfusate. Pacing was maintained during ischemia. LV pressure was monitored throughout ischemia and reperfusion. All hearts ceased to contract within 3 min. The time for LVP to fall to 10% of baseline ( $T_{\text{LVP10}}$ ) was measured to quantify differences in LV function during early ischemia. Mean ischemic  $Ca_i^{2+}$  was calculated as the mean  $Ca_i^{2+}$  recorded from the 2nd through the 14th minute of ischemia. Contracture was defined as an

[000127] To further define the type of NOS enzyme involved in this FGF-2 response, a NOS2-selective inhibitor, L-NIL, was used. Similarly to L-NAME, L-NIL fully inhibited the cardioprotective effects of rFGF-2, significantly reducing  $T_{\rm LVP10}$  (62  $\pm$  3 vs. 124  $\pm$  9 s, L-NIL + rFGF-2 vs. rFGF-2, P < 0.05) and accelerating the onset of ischemic contracture (652  $\pm$  16 vs. 893  $\pm$  7 s, L-NIL + rFGF-2 vs. rFGF-2, P < 0.05). Similarly to perfusion with L-NAME, perfusion with L-NIL alone, in the absence of rFGF-2, did not affect either  $T_{\rm LVP10}$  (67  $\pm$  6 vs. 74  $\pm$  5 s, L-NIL vs. control, P = NS) or the onset of ischemic contracture (740  $\pm$  39 vs. 819  $\pm$  36 s, L-NIL vs. control, P = NS).

Example 4 Efficacy of Intracoronary Versus Intravenous FGF-2 an a Porcine Model Of Chronic Myocardial Ischemia